

February 4, 1965

Mrs. George W. Beadle  
5855 University Avenue  
Chicago, Illinois 60637

Dear Muriel:

I enjoyed hearing from you and will do the best I can to describe the circumstances of the experiment you ask about. I assume you are curious about how we found a copolymer containing adenine and thymine in the absence of added primer, a synthesis we have called de novo formation of dAT polymer. Dr. Howard Schachman, of the University of California in Berkeley, spending his sabbatical year with us at Washington University in St. Louis, Dr. Julius Adler and Dr. I. R. Lehman, postdoctoral fellows, shared in this experiment. Prior to the fall of 1957, we had measured the primed synthesis of DNA using optical and chemical methods. We had run suitable controls but the incubation periods never exceeded two to three hours.

At this time we decided to measure DNA synthesis by a viscometric technique. We expected the synthesis of new DNA to be accompanied by an appropriate increase in the viscosity of the solution. This is indeed what we found. A control was included in which one of the four triphosphates was omitted; fortunately, as it turned out, the omitted triphosphate was dGTP (guanine). Another fortunate circumstance was that measurements in the viscometer were continued up to and beyond four hours. We observed no change in the control tube for the first four hours but then during the next two hours there was an extremely rapid and extensive increase in viscosity. That evening we thought of several possible sources of error: inadvertent addition of the triphosphate (dGTP) which we had intended to omit, bacterial growth, some technical anomaly in that particular viscometer. The next day the experiment was re-run and we were able to eliminate each of these possible sources of error. We established that the development of viscosity occurring after a 4-6-hour lag period took place even though the primer DNA was also omitted. The

day after that we were able to show that all that was necessary to obtain this unprimed reaction were the two triphosphates dATP (adenine) and dTTP (thymine) in addition, of course, to the enzyme and  $Mg^{++}$ . Thereafter it was easy to establish that a high molecular weight copolymer of these two nucleotides (A and T) is the product of this unprimed or de novo synthesis.

As you can see, the experiment was not set up to determine whether synthesis could occur without a primer but rather as a routine control showing the requirement for all triphosphates (A, T, G and C). We had many times before attempted to observe whether synthesis could occur without a primer but had never followed the reaction for as extended a period of time. (It turns out, too, that when taking <sup>samples</sup> at less frequent intervals of time, as one does with isotope incorporation assays, it is relatively easy to miss the synthesis of this copolymer using the relatively crude enzyme preparations available to us at that time. These crude preparations have a nuclease that destroys the polymer rather rapidly.)

This account will diminish your estimation of our insight in finding de novo DNA synthesis. On the other hand, this experience chalks up some points, as if more were necessary, for the scientific discipline of demanding proper controls, including the use of a variety of methods, to check the nature and authenticity of a reaction.

Sincerely,

Arthur Kornberg

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